Biochemical and cellular (liver and kidney) restorative properties of garlic (*Allium sativum*) aqueous extract in cow brain-induced hypercholesterolemic model Swiss albino mice

Swarup Kumar Kundu 1,2, Md. Abu Hadi Noor Ali Khan 3, Shonkor Kumar Das 2

1 Department of Anatomy and Histology, Faculty of Veterinary, Animal and Biomedical Sciences, Khulna Agricultural University, Khulna, Bangladesh
2 Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh
3 Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh

ABSTRACT

Introduction and aim. Garlic is one of the most popular traditional medicinal herbs which has a number of desirable health benefits. The study was designed to depict the improvement of serum biochemical parameters as well as the histomorphological recovery potential of garlic aqueous extract in hypercholesterolemic mice.

Material and methods. A total of thirty Swiss albino mice weighing 24±5g and aged 5 weeks were randomly divided into three groups. Group A: supplied standard mice pellet and water; Group B: standard mice pellet + hypercholesterolemic diet (cow brain: 2 g/kg b.w.t.); and Group C: standard mice pellet + hypercholesterolemic diet (cow brain: 2 g/kg b.w.t.) + garlic extract (25 ml/kg b.w.t.). After four weeks of experimental tenure, samples (blood, liver, and kidney) were collected from each group of mice for serum biochemical analysis and histomorphological study.

Results. Compared with hypercholesterolemic mice, total cholesterol (TC), triglyceride (TG) concentration, and low-density lipoprotein (LDL) levels significantly decreased respectively by 7%, 20% and 48% along with high-density lipoprotein (HDL) levels significantly increased by 47% in garlic extract supplemented group. Based on the histological evaluation in the liver sample of group C, both portal and central veins were normal, and fat droplets were not found in the hepatocytes which were found in the liver of group B. On the other hand, unchanged renal cortex, glomerulus, Bowman’s space, and kidney tubules were seen in group C.

Conclusion. Therefore, the above findings of the present research would assist to provide affirmation about the cholesterol-decreasing and cellular restoration potentiality of garlic aqueous extract.

Keywords. cellular restoration, garlic extract, health beneficial, lipid profile, Swiss albino mice

The list of abbreviations:


Introduction

Liver and kidney are the most essential organs in the body for performing multiple functions like excretion of waste products, hormonal regulation, digestion, and detoxification of harmful drugs. According to the latest World Health Organization (WHO) records released in...
2020, about 1.51% of total deaths were found due to kidney disease as well as 2.94% of total deaths due to liver disease in Bangladesh. Diets rich in highly saturated fat have been identified as one of the major risk factors for developing visceral adiposity like cardiovascular problems, fatty liver syndrome, obesity-related glomerulopathy, etc. Obesity is a burden issue nowadays and the problems, fatty liver syndrome, obesity-related glomerulopathy etc. have been identified as one of the major risk factors for developing visceral adiposity like cardiovascular problems. 

Aim

Therefore, on the basis of the above description main objectives of the present research were to determine:

I. The negative effects of high-cholesterol food (cow brain) on liver and kidney cellular levels and serum biochemical markers.

II. The restorative properties of garlic extract on biochemical profiles as well as the pathological changes of the liver and kidney in diet-induced hypercholesterolemic mice.

Material and methods

Participants and ethical approval

The current research activity was conducted in the Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202. The Ethical Committee of Bangladesh Agricultural University approved the study protocol (AWEBCAU/2019-53). Experimental Swiss albino mice at the age of 5 weeks old were collected from International Center for Diarrheal Disease Research (ICDDR'B), Mohakhali, Dhaka, Bangladesh. The initial weight of the collected mice was recorded at about 24±5 g.

Preparation of garlic aqueous extract

Allium sativum, a plant, is the scientific name for garlic. Its aqueous extract is the source of phenolic compounds which are antioxidant and biologically active. A total of 1 kg of fresh garlic (Variety: BARI Roshun 3) was collected from the Bangladesh Agricultural University horticulture center. Then all the papery skin from the outside of the head of garlic was removed. All the cloves of garlic were separated from each other. Then 250 g of garlic cloves were weighed and crushed in mortar and pestle for 1 min together with 500 ml of distilled water and allowed them standing for 1 h at room temperature. Filtration was done through Whitman no. 4 filter paper to remove the impurities and get up to 100 ml of aqueous garlic extract. Finally, the prepared aqueous garlic extract was taken in a glass container, sealed at its mouth, and stored in the refrigerator for future use.

Study design

Before the commencement of the research, a total of healthy 30 collected Swiss albino mice of either sex were reared in 20x30x10 cm plastic box cages at a standard room temperature (23±2°C), 52±5% relative humidity on a 12 h light and 12 h dark cycle were maintained for 7 days. The rearing boxes were filled with sawdust and changed regularly to maintain hygiene and comfort. At that time mice pellet and fresh drinking water ad libitum were supplied to each mouse. After 7 days of acclimatization, collected Swiss albino mice were randomly categorized into three groups and each group consisted of ten (10) mice.

Group A: Considered as the control group, and given normal mice pellets, and fresh water.

Group B: Cow brain at a dose of 2 g/kg b.w.t as hypercholesterolemic food + mice pellets + fresh water.

Group C: Hypercholesterolemic food (cow brain at a dose of 2 g/kg b.w.t) + garlic extract at a dose of 25ml/kg b.w.t orally + mice pellets + fresh water (Fig. 1)
For the 28 days of research tenure, mice of the control group were supplied standard mice pellets (collected from ICDDR’B) and fresh water. In contrast, cow brain at a dose of 2 g/kg b.w.t was given to both groups B and C of mice as hypercholesterolemic food. Cow brain is a definite source of cholesterol, approximately 100g of cow brain contains 3100mg of cholesterol. Eating cow brains in the daily diet is a natural habit of Asian people, especially Bangladeshi that causes pathological changes in the liver and kidney and enhance total cholesterol (TC) and triglyceride (TG) level. In addition to hypercholesterolemic feed, mice of group C were also given garlic extract at a dose of 25 ml/kg. Garlic has the potential beneficial effects against hypercholesterolemia. About 8-10% of cholesterol can be reduced by the consumption of 1-2 cloves of garlic in a day. Weight of each group of mice was measured on the 1st, 7th, 14th, 21st, and 28th days of the consecutive days of the study period.

**Biochemical analysis**

**Determination of total cholesterol**

The TC was determined after enzymatic hydrolysis and oxidation (CHOD-POD method). The indicator quinoneimine was formed from hydrogen peroxide and 4-Aminophenazone in the presence of phenol and peroxidase. Both reagent and sample were kept at room temperature and mixed 1 ml reagent with 10 µl samples in the test tube. Waited for 10 minutes and placed the mixture in the cuvette. The cuvette was placed in a spectrophotometer at 550 nm and recorded the reading. The reading was calculated by comparing it with the standard value and multiplying it by 200 mg/dL. So the result was expressed as mg/dL (catalogue no. of the used reagent: MO-165218).

**Determination of triglyceride**

The TG was determined after enzymatic hydrolysis with lipase (GPO-POD method). The indicator is a quinoneimine formed from hydrogen peroxide, 4-Aminophenazone, and 4-chlorophenol under the catalytic influence of peroxide. The fatty acid can be hydrolyzed by lipoprotein lipase of an experimental reagent and ultimately produced quinoneimine proportional to triglyceride. This quinoneimine absorbs light of 500 nm. 10 µl samples were mixed with 1ml reagent and kept in a standing position for 15 minutes at room temperature. The mixture was placed in a cuvette of the spectrophotometer and the reading was recorded. This reading was divided by the standard reading and multiplied by 200 mg/dL (catalogue no. of the used reagent: MO-165221).

**Determination of LDL-cholesterol**

This was assayed in each sample by using RANDOX kit (UK). The reagent and sample were kept at room temperature and mixed 0.4 ml precipitating reagent with 0.2 ml sample. After 10 minutes, this was centrifuged at 12000 rpm for 2 minutes. Then the supernatant was separated. This supernatant was used for LDL determination. 50 µl supernatant was mixed with 1 ml cholesterol reagent and stand for 10 minutes at room temperature. This mixture was set in a spectrophotometer by using a cuvette at 500 nm wavelength. The reading was recorded and calculated by comparing standard readings and the fraction was multiplied by 50 mg/dL.

**Determination of HDL-cholesterol**

The HDL cholesterol was assayed in each sample by using RANDOX kit (UK). Low-density lipoproteins (LDL...
and VLDL) and chylomicron fractions were precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol in the HDL fraction, which remains in the supernatant, was determined. The HDL-cholesterol was determined on the principle of estimation of TC (catalogue no. of the used reagent: 1133010).

**Statistical analysis**

All the collected data were analyzed by using Statistical Package for the Social Sciences (SPSS: version 25, IBM, Armonk, NY, USA) software and revealing the results in tabular form. Statistical analysis was performed using one-way analysis of variance (ANOVA). Results were expressed as mean±SE. Differences between groups were considered significant at **p<0.001 and *p<0.05 levels.

**Results**

**Body weight**

The mean body weight of the hypercholesterolemic group (39.9 g) of mice was significantly higher than the control group (31.4 g) of mice. Garlic extract supplementation successfully maintained the average normal body weight in group C (33.1 g) of mice compared to group B.

**Gross morphometric study**

**Liver assessment**

In the current study, no irregular surface, red-brownish color, no detectable pathological lesions, and normal shape of lobes were found in the control group (Group A) of the liver (Fig. 2). Liver become slightly enlarged, and pale yellowish discoloration of the surface was found in the hypercholesterolemic (Group B) group of mice (Fig. 2). In contrast, garlic extract supplementation fully restored the morphological changes in the liver. No detectable abnormalities were seen on the liver surface of group C of mice (Fig. 2). On the other hand, liver weight of the garlic extract-supplemented group was seen as normal compared to the control group of mice but the liver weight of group B of mice was found considerably high (Fig. 3).

**Kidney assessment**

Kidney of the control and garlic extract-treated group of mice showed a normal appearance, brownish-red color, and normal position and shape. No detectable pathological lesions were seen (Fig. 4). Excess peri-renal adipose tissue (PART) accumulated around the lower end of the kidney and the size of the kidney slightly increased compared to other groups of mice (Fig. 4). The weight of the kidney in group C (garlic extract supplemented) of mice was shown normal as the control group of mice (mean ± standard error).
showed normal but hypercholesterolemic feed increased the kidney weight in group B compared to group A of mice (Fig. 5).

**Histopathological study**

Histological analysis was performed by H&E staining for the confirmation of hepatoprotective and renoprotective effects of garlic aqueous extract.

**Hepatoprotective properties of garlic extract**

Histological study of the section of the liver of hypercholesterolemic (Group B) mice exhibited narrowing of the portal vein, hypertrophied hepatocytes, radiated hepatocytes from the central vein and formed coherent groups, merging of the central and portal vein which made it difficult to separate them, Kupffer cells in the hepatic sinusoids, mild to moderate congestion in both central and portal vein, dilated central vein, fat droplets in the hepatocytes (Fig. 6). Surprisingly, following the supplementation of garlic extract to group C of mice liver section showed normal central and portal veins, normal hepatocytes (no fat droplets) as well as congestion was not found in the central and portal veins (Fig. 6). Bi-nucleated hepatocytes were found in every section of the liver.

**Reno-protective properties of garlic extract**

Normal architecture of the cortex and medulla was found in the control group of mice. Due to the garlic extract supplementation, glomerular and tubular fatty infiltration, degenerated renal tubules, and hypertensive glomerulosclerosis was successfully restored in group C of mice (Fig. 7). Glomerulus, Bowman’s space was also seen as normal like the control group of mice (Fig. 7).

**Study of lipid profile**

**Improvement of serum lipid profile by garlic extract supplementation**

In the present study, the mean value of TC concentration increased by 27% in group (B) (179.044±0.412 mg/dL) (Table 1) compared to group (A) (141.296±0.318 mg/dL) (Table 1) of mice. There was a reduction of TC concentration by 7% in group (C) (166.396±0.249 mg/dL) (Table 1) compared to group (B) of mice. TG concentration increased by 72% in group (B) (231.294±0.258 mg/dL) (Table 1) compared to group (A) (115.108±1.067 mg/dL) (Table 1) of mice. However, group (C) (162.312±1.596 mg/dL) (Table 1) showed a decreased value of TG concentration by 20% compared to group (B) of mice. HDL level (78.906±0.417 mg/dL) (Table 1) decreased by 31% and LDL level (46.544±0.203 mg/dL) (Table 1) increased by 197% in group (B) mice compared to group (A) (94.481±0.395 mg/dL & 24.892±0.273 mg/dL) (Table 1) of mice. Surprisingly, group (C) showed a higher
level of HDL (96.06±0.221 mg/dL) (Table 1) at 47% and a decreased level of LDL (33.02±0.465 mg/dL) (Table 1) at 48% compared to group (B) of mice. Statistically, TC, TG, and LDL levels showed a significant **p<0.001 increase in group B (Fig. 8) compare to control group A, as well as a significant **p<0.001 reductions seen in HDL level of group (B) of mice (Fig. 8). In contrast, garlic extract supplementation (group C) significantly **p<0.001 reduced TC, TG, and LDL concentration and significantly **p<0.001 increased HDL concentration compare to group B (Fig. 8). In addition, the concentration difference of TC, TG, and LDL between the control (group A) and group C was significant at the level of *p<0.05 and LDL concentration was almost similar in both the A and C groups of mice (Fig. 8).

**Fig. 8.** Diagram represents the analysis of the protective effects of garlic extract in lowering the serum lipid profile. TC – total cholesterol; TG – triglyceride; HDL – high-density lipoprotein; LDL – low-density lipoprotein. Results were expressed as mean ± standard error. (**) indicates a statistically significant difference at the level of **p<0.001; (*) indicates a statistically significant difference at the level of *p<0.05 from the control (group A) of mice

<table>
<thead>
<tr>
<th>Parameters (mg/dl)</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, mg/dl</td>
<td>141.296±0.318</td>
<td>179.044±0.412**</td>
<td>166.396±0.249*</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>231.294±0.258**</td>
<td>231.294±0.258**</td>
<td>162.312±1.596*</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>78.906±0.417**</td>
<td>78.906±0.417**</td>
<td>96.06±0.221</td>
</tr>
<tr>
<td>LDL, mg/dL</td>
<td>46.544±0.203**</td>
<td>46.544±0.203**</td>
<td>33.02±0.465*</td>
</tr>
</tbody>
</table>

* (***) and (*) denotes a statistically significant difference at the level of **p<0.001 and *p<0.05 respectively

**Discussion**

The present study showed that consumption of a cholesterol-rich diet (cow brain) alters the gross and histarchitecture of the liver, which is in complete agreement with the results of Korish and Arafah. It was reported that regular high-cholesterol feed consumption causes excess energy intake, growing obesity, and a tendency to inactivity. Liver weight was significantly increased compared to the control group. Liver become pale and showed slight hepatomegaly, degenerated hepatocytes, and fatty droplets in the hepatocytes.16 Kundu et al. reported similar results in their study where cow brain was used as a cholesterol-rich diet.67 Our study exhibited that garlic extract feeding surprisingly restored gross and cellular changes in the hypercholesterolemic liver. Garlic extract supplementation eliminates fat droplet accumulation in hepatocytes and reduces organ weight. The study conducted by Zhang et al. also agrees with our statement. They found a significant reduction in N-nitrosodimethylamine-induced liver weight, surface nodule, and liver enlargement, and improvement in the hepatocellular architecture from hyperplastic nodules and destroyed hepatocytes after garlic oil application during the study period.77 Tran et al. also speculated in their research, the aqueous extract of garlic reduced the weight and restore the texture of the CCl4-intoxicated liver, and reduced the proliferation of mononuclear cells around the hepatic veins.18 In addition to this, our current research showed an accumulation of PART, a slight increase in kidney weight, and several fatty changes in the cow brain-induced hypercholesterolemic kidney tubules and glomerulus. In the previous study conducted by Salim et al. stated that a high-fat diet leads to hypercholesterolemia and disturbance of lipid profile, impaired kidney function, and accumulation of fatty droplets in the glomerulus and kidney tubules. There is a close relationship between TG concentration and fat deposition in organs. A higher level of triglyceride is the key factor for the deposition of fat in organs.19 Increased hypercholesterolemia alters the cellular structure of the tubules and deposits fatty droplets in the tubules that ultimately inhibit cell metabolism and impede filtration.20 Alansari et al. were also in agreement with our current finding. Tubular deformities, atrophied glomerular capillaries, and vacuolar degeneration in tubules frequently appear due to a high-fat diet.21 But garlic extract supplementation mitigated the cellular changes induced by a high-fat diet and completely restored fatty infiltration in both tubules and glomerulus and ultimately improved filtration capacity in the current findings. El-Shenawy and Hassan also revealed a statement in a study that garlic extract improved the cellular alteration of kidneys induced by a hypercholesterolemic diet as well as recovered the damages from fatty infiltration in renal tubules.22

From the biochemical analysis of the present study, it has been stated that TC and triglyceride TG concentration was significantly reduced by garlic extract consumption. However, this statement was in agreement with Ali and Thomson who mentioned that administration of garlic to hypercholesterolemic rats, humans, and cell cultures is effective in decreasing TC and TG concentration.23 Sher et al. also observed similar findings. They described in the study, levels of TC (32.8±0.7 mg/dL) and TG (44.0±0.9 mg/dL) concentration significant...
Aouadi et al. The protective mechanisms of the ben-

percholesterolemic rat caused a significant rise in HDL

current study, feeding garlic extract to an induced hy-

decreasing TC and TG reported by Katsuki et al. and

suppressing LDL oxidation, increasing HDL as well as

effective effects of garlic in CVDs may be achieved by

administration suppressed LDL oxidation and increased

HDLC which may be one of the beneficial effects of garlic

in cardiovascular diseases. In our present study, garlic

extract supplementation significantly reduced LDL

levels which were also revealed in the study of Shela et al.,

Merat and Fallahzadeh, Aslani et al, and Yeh et al. They

observed that garlic administration in rats suffering

from hypercholesterolemia induced by a high-cho-

lesterol diet significantly reduced LDL levels. Therefore, according to the above de-

scription, it can be suggested that garlic extract has a

protective function to restore serum biochemical pa-

rameters and to restore the ability of gross and histo-

morphological changes of organs (liver and kidney) in

hypercholesterolemia induced by a cholesterol-rich diet.

Conclusion

Cholesterol-rich food intake has several adverse health

effects including changes in lipid profile, changes in or-

gan weight, fat accumulation in various organs, especially

the liver and kidney, various pathological lesions in gross

and microscopic aspects, etc. In our society, people are

not aware of the tremendous beneficial effects of medic-

inal herbs like garlic. In the present study, garlic extract

supplementation restored the lipid profile, excess organ

weight, and histopathological lesions of the liver and kid-

ney, specifically removing fat deposits in the cells. How-

ever, further studies on a molecular basis are needed to

investigate the pathophysiological mechanism of garlic

extract in restoring pathological changes as well as elevat-

ed lipid profiles in hypercholesterolemic mice.

Acknowledgments

The authors are highly grateful to the Department of

Anatomy and Histology, Faculty of Veterinary Science,

Bangladesh Agricultural University, Mymensingh-2202 as

well as the university central laboratory for the tech-
nical support. Special thanks to MoST (Ministry of Sci-

cence and Technology), Bangladesh for providing the

NST (National Science and Technology fellowship as fi-

nancial support to conduct the research efficiently.

Declarations

Funding

MoST (Ministry of Science and Technology) Bangla-

desh; NST (National Science and Technology, 2018-

2019) (NST SL No. 303 and ID No. 4110).

Author contributions


Conflicts of interest

Nothing to disclose.

Data availability

Datasets analyzed during the present study and/or are

available from the corresponding author upon reason-

able request.

Ethics approval

The Ethical Committee of Bangladesh Agricultural

University approved the study protocol (AWEEC/BAU/2019-53).

References

1. Lonardo A, Mantovani A, Targher G, Baffy G. Nonalcoho-

lic Fatty Liver Disease and Chronic Kidney Disease: Epi-

demiology, Pathogenesis, and Clinical and Research Imp-


2. World Health Rankings site. https://www.worldlifeexpect-

ancy.com/country-health-profile/bangladesh. Accessed

February 20, 2023.

3. Ix JH, Sharma K. Mechanisms linking obesity, chro-

nic kidney disease, and fatty liver disease: the roles of


4. Kundu SK, Khan MA, Das SK. Cow brain consumption

causes hypercholesterolemia: An in vivo study. Asian Jour-

nal Of Dairy And Food Research. 2021;40(2):177-180. doi:

10.18805/ajdfr.DR-216

5. Bengal Meat Beef Brain: https://jarlimited.com/product/ben-


6. Nessa MU, Rahim AT, Kabir Y. Fatty acid profile of fast fo-

ods, traditional snacks and sweetmeats of Bangladesh. KU


